

EXHIBIT DX2

TO DECLARATION OF PETER J. GOSS IN
SUPPORT OF DEFENDANTS' OPPOSITION TO
PLAINTIFFS' MOTION TO EXCLUDE THE
OPINIONS AND TESTIMONY OF
JIM HO, PH.D

Expert Report of Jim Ho, Ph.D.

In re Bair Hugger Forced Air Warming Products Liability Litigation

My name is Jim Ho. I spent my career as a researcher with Defence Research and Development Canada, an agency of Canada's Department of National Defence. I dedicated most of my research to the study of airborne microbes. I have been retained by 3M to provide expert testimony on the microbiological issues raised by the plaintiffs' allegations that the Bair Hugger patient warming system increases the risk of surgical site infections.

I. Education, Training, and Experience

My education, training, and publications are detailed in my bio, attached as Exhibit A, and my publications are listed in Exhibit B. My early education took place at Malacca High School, Malaysia and St. Joseph College in Hong Kong. I came to Canada to study at McGill University in Montreal where I obtained a BSc and MSc in microbiology. After obtaining my PhD in microbial biochemistry from the University of Kentucky, I joined the Canadian Department of National Defence in the early 80's. My broad mandate was to develop technologies for the Canadian Forces (CF) to detect biological warfare (BW) agents. My career can best be summarised chronologically as pre-1990 and post-1990 activities, coinciding with the deployment of equipment for real time detecting biological threats in the first Gulf war. During the first period, my work involved understanding biological aerosol characteristics, while the latter period dealt with developing a robust biological detection system for the Canadian military and other friendly clients.

Biological detectors developed by the US in the 70's and 80's were based on wet chemistry to measure a single parametric "biological component" as detection criteria. These machines failed due to the unrefined measurement principles employed causing false alarms or just didn't work reliably. From these lessons, I formulated a two-step approach to biological detection: A. rapid front end based on optical light scattering technology and B. slower back end system based on generic UV or fluorescent dye based wet chemistry using variations of existing antibody binding principles. The significance of the scheme is that the optical front end can operate continuously without requiring user attention while the wet chemistry back-end only needs to run on trigger, thus conserving expensive reagents and other resources. Just as important, the rapid responding front end will provide timely information to the military commander for when to initiate protective posture, e.g. donning of respirators. As antibody based clinical methods were relatively well developed and accepted at the time, the major knowledge gap was determined to be in optical methods for differentiating live biological aerosols from background material.

Two critical discoveries were made during the early studies. Firstly, it was found that artificial biological aerosols generated as significant threats contained particles mostly in the 2-10 μm size range and secondly, by using a commercial light scattering instrument, these could be distinguished from background aerosols that predominated in the 0.5-1 μm region. Later, it was discovered that biological aggregates depolarised light

significantly and this characteristic could be used in remote detection in LIDAR systems (related to RADAR but using laser light). In the middle 1980's, I led a team of scientists from Defence Research & Development Canada (DRDC) at Valcartier and DRDC Suffield in a series of fundamental LIDAR studies on biological aerosols. Knowledge gained from this period significantly influenced fluorescence-based long range standoff biological detection directions for Canada and her NATO allies.

When the Gulf war broke, BW detector systems were deployed at CF locations on Qatar and Bahrain developed from my two step approach, using light scattering techniques and custom software for bioaerosol detection. Canada was the first to deploy such technologies during wartime applications. For having been to the war zone to deploy and train the CF users, my team and I were awarded campaign medals by the Canadian and Saudi governments, a historic first for Canadian civilians. Within multinational agreements, these methodologies were freely shared with allies. Indeed, the two step approach has become accepted as doctrinal for most countries with active BW detection programs.

After the gulf war, in the early 1990's, while searching for better ways to characterise biological particles, I discovered that a single spore (typical of anthrax threats), excited with 340-360 nm UV light, could be induced to fluoresce at wavelength regions representative of important biological metabolic molecules like NADH and riboflavin. As these substances could be related to respiratory chain reactions, such fluorescence signals were speculated to reveal important live biological characteristics and thus mere sand particles would not interfere with bioaerosol measurements. Under my leadership, with the help of outside contractors, my team proceeded to build the first fluorescence aerodynamic particle sizer (FLAPS1) for real-time detection of biological aerosols. The success of the instrument was demonstrated at a joint field trial in 1995 where many countries gathered at Dugway Proving Ground in Utah to compete at biological detection in a program hosted by the US Joint Program Office. The Canadian team, which I led, scored first in detection performance. A second-generation instrument was built with better physical attributes (FLAPS2) and with this instrument, Canada again came in first during the 1996 joint field trial. Success was repeated in 1997 with a new integrated system that had sample collection built-in. During this time, I also directed the implementation of custom alarm algorithm software with help from contract programmers.

In the later 1990's FLAPS2 technology was used to manufacture a trigger collector system that served as CB sentry for the Canadian Forces. It was deployed in Operation Determination on HMCS Toronto during the Persian Gulf crisis in 1998. In addition, DND has purchased these biodetection systems and are permanently installed in Canadian frigates that patrol hostile regions of the world. The US Army became the biggest customer of the FLAPS instrument. I was lead scientist in designing a third generation (FLAPS3) that became commercially competitive, with world wide sales to NATO and other friendly countries. This product, launched in 2004 was the outright winner of the Technology Readiness Evaluation (TRE) field trials held in June 2003 at Dugway, Utah.

II. Fundamentals of Aerobiology

Goodlow and Leonard (1961) were aerobiologists who worked at the U. S. Army Chemical Corps in Fort Detrick, Frederick, Maryland, one of the best funded and respected of such research institutes. During a conference on bacterial survival in air, they mentioned that microbial viability is considered important for replication under experimentally conditions. Also that viability appeared essential for infectivity but not all cells that are viable are infectious. It was noted that some bacteria were inherently aerosol-stable, for example staphylococci, mycobacteria, and *Bacillus anthracis* spores. Larger airborne particles of *B. anthracis*, *Pasteurella tularensis*, *Coxiella burnetti*, *Brucella suis* were more infective than smaller ones, the critical size range being 1-5 μm (note that native single cells are about 0.5-1 μm). Similarly, larger particles were more resistant to sunlight exposure. What puzzled them was that *Escherichia coli* was more air sensitive than *Staphylococcus citreus* and *Staphylococcus albus*.

While on the topic of infectivity, it may be useful to discuss the source of infectious particles in hospital environments. Thompson et al. (2014) estimated bacterial aerosol generation from humans performing activities in a chamber. They found that hair length correlated with the numbers of *S. aureus* colony-forming units disseminated during these activities. Their results confirm previous observations that persistent carriers of *S. aureus* among hospital personnel have been implicated in nosocomial (acquired in a hospital) transmission (Lidwell et al. 1966). Cimolai (2008) surveyed the literature on MRSA (drug resistant *S. aureus*) persistence in hospitals and concluded that humans were the prime sources of infective material. He concluded with this astute quote: “The spread of MRSA from patient to the environment was at one time storied to the author by a wise and retired nurse whose broad experience included traineeship in the UK, employment in the US, and retirement in Canada. She likened the patient to an automated popcorn maker and the MRSA to the popcorn kernels. Without a popcorn maker, there would be no popcorn spilled into the environment. The more kernels placed into the machine, the more likely that popping kernels would be spilled into the environment and outside of containment. When the machine was removed or depleted of kernels, there was a lesser probability of kernel dissemination. From time to time, a less than optimum cleaning of the counter or floor near the popcorn maker, even after its removal, would lead to the discovery of residual kernels. The more efficacious and repetitious the environmental cleaning, the less likely that kernels would be found. Some of these stray kernels, if re-entered into the popping apparatus, might thereafter pop and spill again. While not a perfect analogy, the implications seemed quite obvious and sensible.”

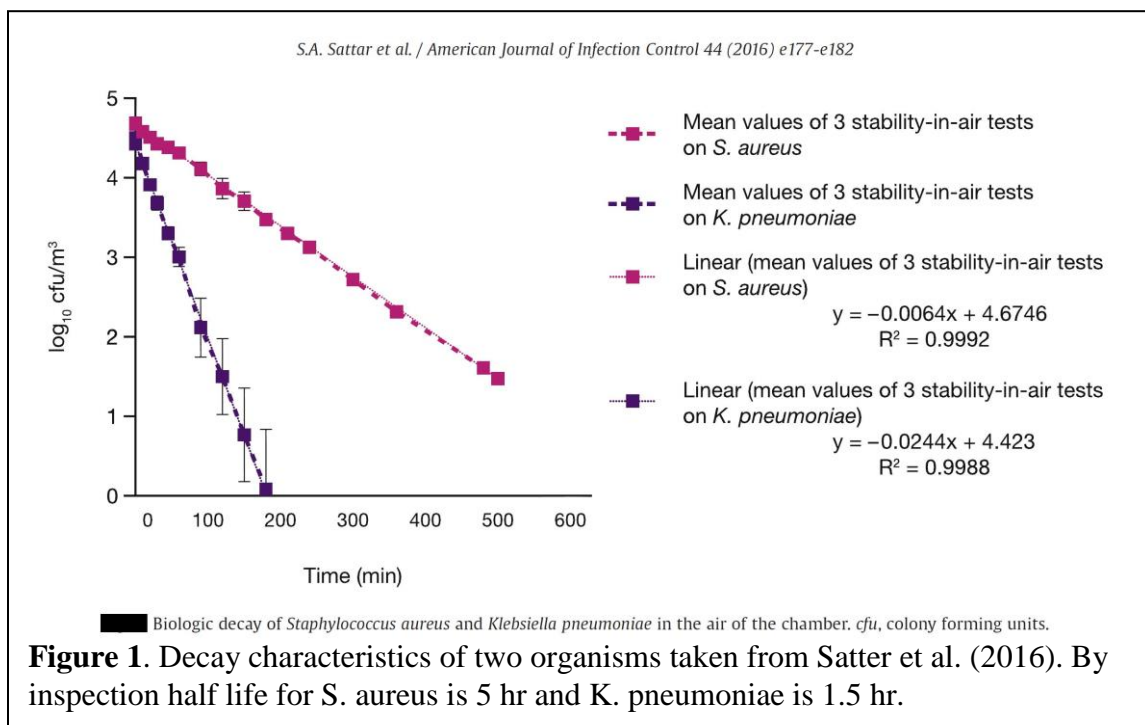
Apart from minimal nutrient concentrations, the amount of free water is essential for microorganisms to survive in dry air environment. The condition is described as water activity, the ratio of the vapor pressure of water in the substrate to the vapor pressure of free water (Cole and Cook 1998). For this reason, as will be discussed later in this narrative, some form of encapsulation to protect the cell membrane will be very helpful.

Chemical insults can also affect aerosol survival. Oxidative conditions that degrade cell membrane are the next important factor. For example, in the presence of

highly reactive oxygen species or peroxidation in *Mycobacterium lepraemurium*, ordinarily a very robust cell with thick waxy encapsulation, the organism suffered drastic changes in the lipid envelope (Wek-Rodriguez et al. 2007). Consequently the reaction killed the bacteria and abolished their ability to produce infection in the mouse.

Vegetative cells have fragile cell walls so are more susceptible to external perturbations. Spores on the other hand possess a very tough wall reinforced with calcium conferring it with high resistance to drying, harsh chemicals and UV rays (Driks and Eichenberger 2016).

For reasons explained by Romero (2016), it is rare to encounter single organisms in nature. However, in the laboratory, using very specialized equipment, it is possible to generate mostly single cells floating in air for survival studies. For example, in 2 minutes, survival was only 0.1% for single cell *Escherichia coli* in aerosol at 85% humidity (Cox 1968). In this work, the particulate population was contained in a large rotating steel drum to slow down settling while samples were extracted for analysis. In contrast, in measurements made without similar facilities, it is highly unlikely that single cell status can be achieved. Nevertheless, Domon et al. (2016) studied *Staphylococcus aureus* survival in the open air on surfaces. Their findings demonstrated that 70% of the *S. aureus* isolates, including the MRSA strain, became undetectable at 12 h in dry air, while the others remained viable for up to 24 h. They suggested that it is difficult for MRSA organisms to survive on dry surfaces found in public areas. Figure 1 shows the survival characteristics of two vegetative organisms. *S. aureus* appears to decay slower than



Klebsiella pneumoniae in air (Satter et al. 2016). Of the four genera used in their study

Marthi et al. (1990), it was found that *Erwinia herbicola*, naturally encapsulated in the polymer levan, showed the highest degree of survival during aerosolization over distances of 15 m. There was no significant difference in the survival of *Enterobacter cloacae*, *K. planticola*, and *P. syringae*. The survival strategy appears to be different for a variety of organisms dependent on its natural habitat (Stanley-Wall et al. 2015).

As a historical account, Davies and Noble (1962) provided this quote of the association of bacteria with skin particles: “That bacteria can be recovered from the skin has been reported by many workers; moreover, in man, a complete layer of skin is desquamated every one or two days (Rothman 1954). Skin scales were found in the air of hospital wards and barracks as early as 1855 (Thomson 1855, Rainey 1855, Hewlett et al. 1861, Temple-Wright 1869, de Chaumont 1867). More recently Davies (1958) observed that the number of skin scales in the air was increased during bedmaking; and the possibility that bacteria are carried on scurf and skin scales was suggested by Gordon (1905) and Allison and Gunn (1932). It seemed reasonable, therefore, to examine the air and dust of hospital wards to determine whether skin scales were present and whether viable bacteria could be detected on them.”

Characterizing bacterial aerosol in the hospital air was a novelty in the early 1960s. Davies and Noble (1963) were the first to observe that culturable *S. aureus* aerosol particles originated from human skin. Previous to that, Lidwell et al. (1959) showed that, on average, these particles each carried about 4 viable units. These results suggest that the bacteria were not suspended freely in the air but were carried on other, larger particles. Subsequently Noble (1961) reported that the mean equivalent diameter of air-borne particles carrying staphylococci was estimated at 13.5 μm .

As these were the early days of aerobiology, estimating complex particle size in air was a novelty. Noble (1961) consulted with the inventor (Bourdillon et al. 1941) of the slit sampler to figure this out using aerodynamic principles. Here is a quote from his paper: “The equivalent diameter of a particle is the diameter of a sphere of unit density which has the same settling-rate in still air (calculated on the basis of Stoke’s Law) as the particle in question (Bourdillon et al. 1948).” For over 8 decades, the slit sampler was the gold standard for measuring culturable biological aerosol particles with respect to time and air volumetric concentration.

The characterization of skin related biological particles has been brought up to modern day methods via the FLAPS technology. In their paper, the authors used an instrument labeled UVAPS, a trade name for FLAPS (Bhangar et al. 2016). They reported that human skin was a source of coarse particles that showed highly fluorescent signals and that the aerosol particle size distribution was of 3–5 μm diameter range. In this case, the authors implied that the fluorescent particles have viability characteristics. Based on flow cytometry work done to demonstrate the connection between intrinsic fluorescence and cell viability (Laflamme et al. 2005), it has become common for present day workers to make this assumption.

In any discussion on outer bacterial encapsulation, different terms may be encountered to describe the exopolysaccharides (EPS). As shown in table 1, dependent on the sugar complex, different specific terms may be used in place of EPS, for example levan macromolecules are made up of mostly fructose. The generic term “biofilm” came

EPS	Components	Substituents	Applications
Alginate	GulA, ManA	Ace	Food, feed, medicine, research
Cellulose	Glc		Food, medicine, acoustics
Colanic acid	Glc, Fuc, GlcA, Gal	Ace, Pyr	N.a.
Curdian	Glc		Food, cosmetics, medicine, construction chemistry
Dextran	Glc		Medicine, chromatography
Diutan	Glc, Rha, GlcA,	Ace	Construction chemistry,
Gellan	Glc, Rha, GlcA	Ace, Gly	Construction chemistry, food, feed
Hyaluronic acid	GlcA, GlcNAc		Medicine, cosmetics
Levan	Fru, Glc		Food (prebiotic), feed, medicines, cosmetics, industry, glue
Succinoglycan	Glc, Gal	Ace, Pyr, Suc	Oil industry, cosmetics
Welan	Glc, Rha, GlcA, Man	Ace	Construction chemistry,
Xanthan	Glc, Man, GluA	Ace, Pyr	Food, feed, technical applications, oil drilling

Table 1. Overview of the most relevant bacterial exopolysaccharides (EPS). Component legend: Glc, glucose; Rha, rhamnose; Fuc, fucose; Fru, fructose; Gal, galactose; Man, mannose; GlcA, glucuronic acid; ManA, mannuronic acid; GulA, guluronic acid; GalA, galacturonic acid; GlcNAc, N-acetyl-glucosamine; Pyr, pyruvate; Ace, acetate; Gly, glycerate; Suc, succinate (Schmid et al. 2015).

to popular usage starting in the early 1980s and does not denote a specific chemical makeup. Interestingly, over time, many authors writing on the subject gave the wrong impression that Bill Costerton had coined the term. For example, Prakash et al., (2003) wrongly credited Bill Costerton for coining the term “biofilm” in the 1978 Scientific American article. At that time Costerton's group had used to term “glycocalyx” to describe extracellular polysaccharide. In 1981, Costerton adopted the term in his paper titled “Observations of Fouling Biofilm Formation.” It was in 1976 that Williamson and McCarty (1976) described a model to explain how chemicals move in and out of biological film “biofilm.” Matson and Characklis (1976) studied the diffusion characteristics of biofilm, referencing genesis of the term to Williamson and McCarty (1973) in an oral presentation and abstract at a conference in Cincinnati. Many years later, in a telephone conversation with Williamson, it was revealed that the term was

coined to shorten “biological film” during writing of his PhD thesis in the early 1970s as a time saving measure.

Staphylococcus aureus and *Staphylococcus epidermidis* produce adherent biofilms containing the capsular polysaccharide-adhesin (PS/A). This sticky substance mediates cell adherence to biomaterials. Biofilm cells also produce another complex molecule, polysaccharide intercellular adhesin (PIA) made up of 28 kDa soluble linear beta(1-6)-linked N-acetylglucosamine (Maira-Litran et al. 2002).

In a review paper, Limoli et al. (2015) outlined the relationship of bacterial extracellular complexes with the potential benefits that can occur for bacterial organisms. In general, bacteria produce a capsular surrounding (EPS or biofilm) matrix consisting of a mixture of proteins, extracellular DNA, and polysaccharides (complex sugars) that is integral in the formation of bacterial communities. The polysaccharide component of the matrix can provide many diverse benefits to the cells in the biofilm, including adhesion, protection, and structure. Polysaccharides act as molecular glue, allowing the bacterial cells to adhere to each other as well as surfaces. Adhesion facilitates the colonization of both biotic and abiotic surfaces by allowing the bacteria to resist physical stresses imposed by fluid movement that could separate the cells from a nutrient source. Polysaccharides can also provide protection from a wide range of stresses, such as desiccation, immune effectors, and predators such as phagocytic cells and amoebae. Finally, polysaccharides can provide structural layers, allowing stratification of the bacterial community and establishing gradients of nutrients and waste products. This can be advantageous for the bacteria by establishing a heterogeneous population that is prepared to endure stresses created by the rapidly changing environments that many bacteria encounter. The diverse range of polysaccharide structures, properties, and roles highlight the importance of this matrix constituent to the successful adaptation of bacteria to nearly every ecological niche. In addition to these conventional benefits, more recently, Dragos and Kovacs (2017) also included other functions for the extracellular matrix; for example participation in cell to cell signaling, migration, and genetic exchange either being freely shared with other species or being exclusive to siblings.

The relevance of the current discussion is to make aware the importance of biofilm to bacterial adherence to surgical surfaces. Garcia-Gareta et al. (2016) summarized recent knowledge in bacteria adherence, colonization and development into biofilms on the surface of biomaterials causing infections with distinctive characteristics. At the same time, biofilms appear to alter protection from the host immune system and an increased resistance to antibiotic therapy in comparison to their free floating cells.

Apart from skin derived biological particles discussed above, bacteria and viruses that originated from having infected humans are frequently shed via numerous mechanisms, for example coughing. Under such conditions the organisms travel in air as dehydrated aggregates. Recently Zhou et al. (2016) studied avian influenza viruses in air using a NIOSH bioaerosol sampler. They reported that although individual organisms are submicron RNA units, the viable viruses were identified predominantly from particles > 4 µm, occasionally from 1–4 µm and none from particles < 1 µm. Due to aerodynamic

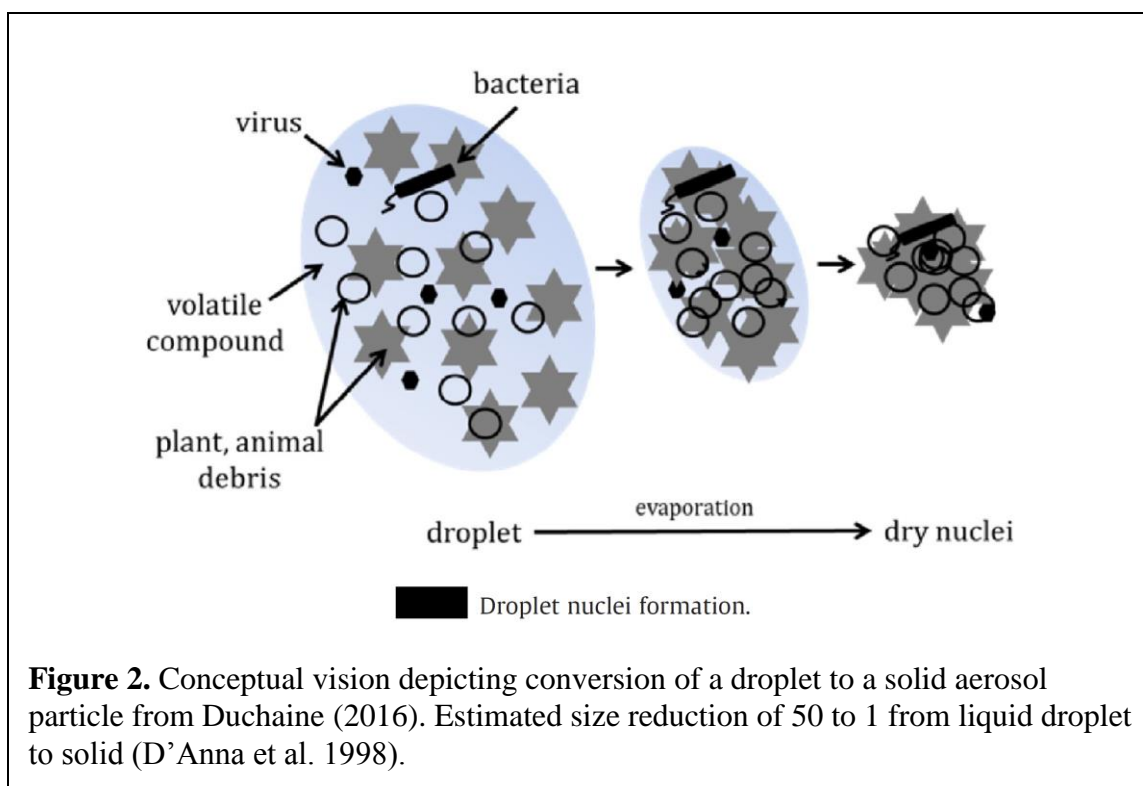
considerations, similar assumptions can be made for bacterial particles generated in nature.

Taylor and Leeming (1993) employed a virtual impactor as previously described by Davies and Noble (1962) to estimate the size of skin squames. It was determined that the size of bacteria carrying particles as between 4 μm and 20 μm . Previous to that, Mackintosh et al. (1987) measure the dispersal of bacteria-carrying particles during exercise among male and female subjects. They reported particle size range between 5 μm to about 20 μm with 7-10% less than 10 μm . It appears that both groups had comparable size estimates.

As shown in Table 1, nature has provided bacteria with the ability to make a variety of extracellular polymers called exopolysaccharides (EPS) with a variety of sugars. Summarized are different kinds of EPS that are commonly associated with bacterial sources along with their commercial applications. For this discussion, the important functional characteristic is that EPS confer protection for survival during hostile conditions. For example, biofilms are gel-like multicellular microbial communities encased in a slimy extracellular polymeric matrix (EPS matrix) that protects them from harmful environmental conditions (Davey et al. 2000).

“Microorganisms synthesize intracellular, structural and extracellular polymers also referred to as biopolymers for their function and survival. These biopolymers play specific roles as energy reserve materials, protective agents, aid in cell functioning, the establishment of symbiosis, osmotic adaptation and support the microbial genera to function, adapt, multiply and survive efficiently under changing environmental conditions” quoted from Vijayendra and Shamala (2014). The major carbohydrate component of the *B. subtilis* EPS matrix is either polysaccharide EpsA-O, which is composed of glucose, galactose, and N-acetylgalactosamine, or polysaccharide levan (β -fructan) (Benigar et al. 2016). Due to their protective structures, in clinical microbiology, EPSs are major issues with respect to drug therapy (Davey and O’toole 2000). However, one way that nature has evolved to penetrate EPS layers involved specific enzymes produced by bacterial viruses (Latka et al. 2017). In the quest for non-antibiotic based *S. aureus* eradication, phage therapy is the hottest topic in microbiological research. In a study, sheep sinus was infected with clinical *S. aureus* organisms. A mixture of different Staph specific viruses achieved 85% lysis success with no side effects (Drilling et al. 2017).

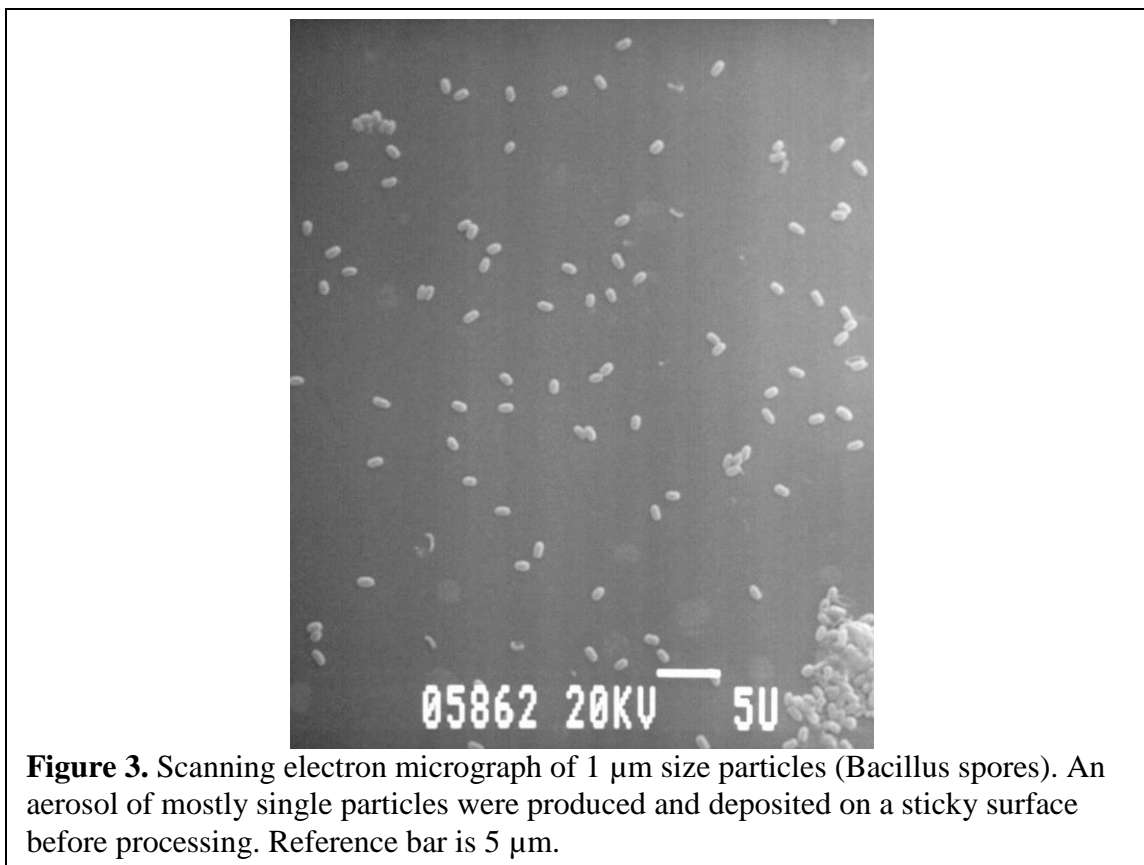
In a review on biological aerosols, Duchaine (2016) depicted the evolution of a liquid droplet containing a variety of microbial agents to a solid aggregate particle (figure 2). This has been a conceptual vision for many years but it is possible to predict some aerosol size characteristics using published data. For example, using a special box and glass slides, Xie et al, (2009) measured droplets emitted from humans during talking and coughing. They reported drop size range of 50-100 μm . Droplet size can be converted to



solid particles by using a factor of 50 to 1 from liquid droplet to solid as suggested by D'Anna et al. (1998). The resultant particle size range works out to be 1-20 μm . If this particle distribution were to persist for any length of time, some of the larger material may settle out rapidly leaving a distribution of 1-10 μm remaining aerosolized. In a recent study, Lui et al. (2017) developed a model to predict conversion of liquid droplet to dry particles. They claimed that droplets at 0% RH ambient conditions, 60 μm droplets would dry out and become particle nuclei with a diameter of 19 μm . This appears to provide close proximity to that what Duchaine (2016) attempted to depict.

Thus it is possible to expect that indoor pollution can be a serious issue given the presence of suitable contaminating agents. Recently Ijaz et al. (2016) reviewed a variety of microorganisms that can potentially become indoor pathogens that spread via the aerosol route. The examples they gave were *Staphylococcus* spp, especially MRSA (Methicillin-resistant *Staphylococcus aureus*), *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Clostridium difficile*, *Bacillus anthracis*. Similarly, it can be postulated that such conditions also occur in hospital operating rooms (OR). If the conditions outside an OR were contaminated with any of these bacterial pathogenic aerosol particles, Kalliomaki et al. (2016) predicted that passage of personnel through doors would introduce significant contaminants. This observation was later confirmed by independent measurements using sonic anemometers by a Spanish group (Villafruela et al. 2016). Another significant source of OR aerosol contamination is derived from the use of electrosurgical tools (ESTs) in tissue cauterization as reported by Romano et al. (2017).

Given the above discussion on EPS and the tendency for biological organisms to stick together as clusters, it is clear that to produce single aerosol particles of individual bacterium or virus is very difficult. Traditionally, in laboratory experiments, a Collision generator had been used to provide single particle bacterial aerosol as shown in figure 3.



In this example single *Bacillus* spores were deposited on a sticky surface then observed via scanning electron microscopy (SEM, unpublished). Interestingly, for many years, aerobiologists have used the Collision generator to produce *Escherichia coli* aerosol to study its decay characteristics in air. It took over 40 years for workers to demonstrate that the Collision generator itself has a tendency to damage membrane integrity, compromising cell viability as described by Thomas et al. (2011).

As most workers in aerobiology have accepted that single particle aerosol is not representative of natural biological presentation, modern methods have been employed to produce a variety of larger particle size ranges. Currently, Gut et al. (2016) employed a specialized instrument to produce 5 μm aerosol particles from a liquid culture of *Yersinia pestis*, the cause of plague (figure 4). They used a syringe-fed (Cole-Parmer, Vernon Hill, IL) 120-Hz ultrasonic generator (Sono-Tek, Milton, NY).

Donnison et al. (2004) studied bacterial survival of *Serratia entomophila* and spores of *Bacillus subtilis* var. *niger* (BG) in outdoor conditions. For large volumes, they used a high-pressure horticultural sprayer to maximize aerosol production and a low-

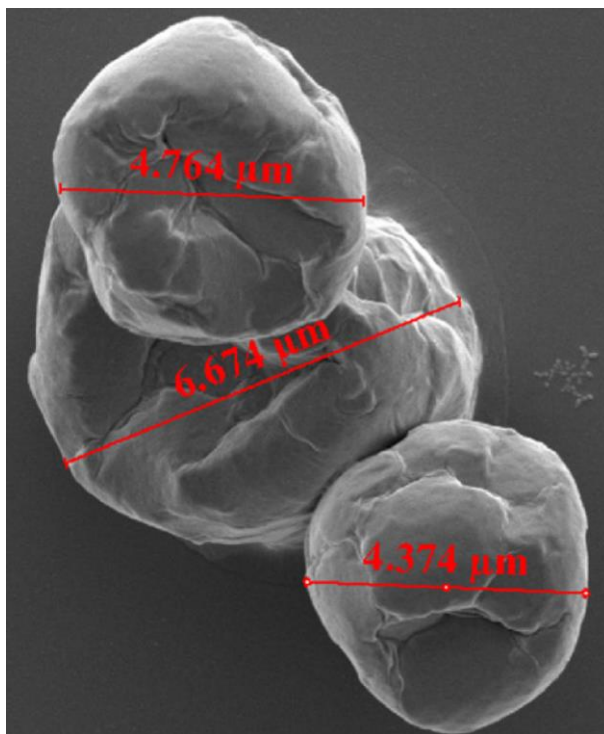


Figure 4. An SEM picture of a typical biological particle aggregate produced with a SonoTek generator. From Gut et al. (2016)

pressure rotary sprayer for lower concentrations. Aerosol particles were collected with impaction on conventional agar growth media. It was observed a greater decrease in survival for *S. entomophila* compared to BG. It could be interpreted that BG apart from being a hardy spore, survived better due to levan encapsulation (Oner et al. 2016).

Using conventional agar growth plate methods for estimating live biological particle concentrations in air has been the gold standard for 24/7 monitoring but it is expensive and time consuming (Bourdillon et al. 1941). For this reason, a few workers have attempted to use light scattering optical instruments as a surrogate for alerting the presence of live or infectious particles. Historically, the first such failed attempt (Gucker et al. 1947) was initiated by the US military for detecting anthrax aerosol.

Stocks et al. (2010) compared particle light scatter measurements with growth on agar plates in an operating room, as illustrated in figure 5. Their results did not find correlation between the two methods as illustrated in this author's quote: "The precision of predicting CFU/m³ counts from particulate count was limited. The 95% prediction interval for CFU/m³ count (ie, after back-transformation of sqrt_CFU/m³) ranged from 612 CFU/m³ at low (<2000) 10 μm particles/m³ to 632 CFU/m³ at high (>8000) 10 μm particles/m³." It is of interest to note that the live particle counts appeared unusually high and this was due to the original numbers being transformed by a square function.

Landrin et al. (2005) wanted to discover if particle counting with a commercial light scatter instrument could be predictive of microbiological contamination of air in

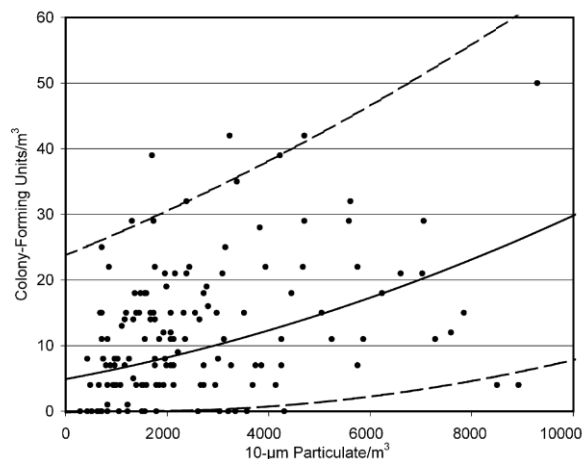
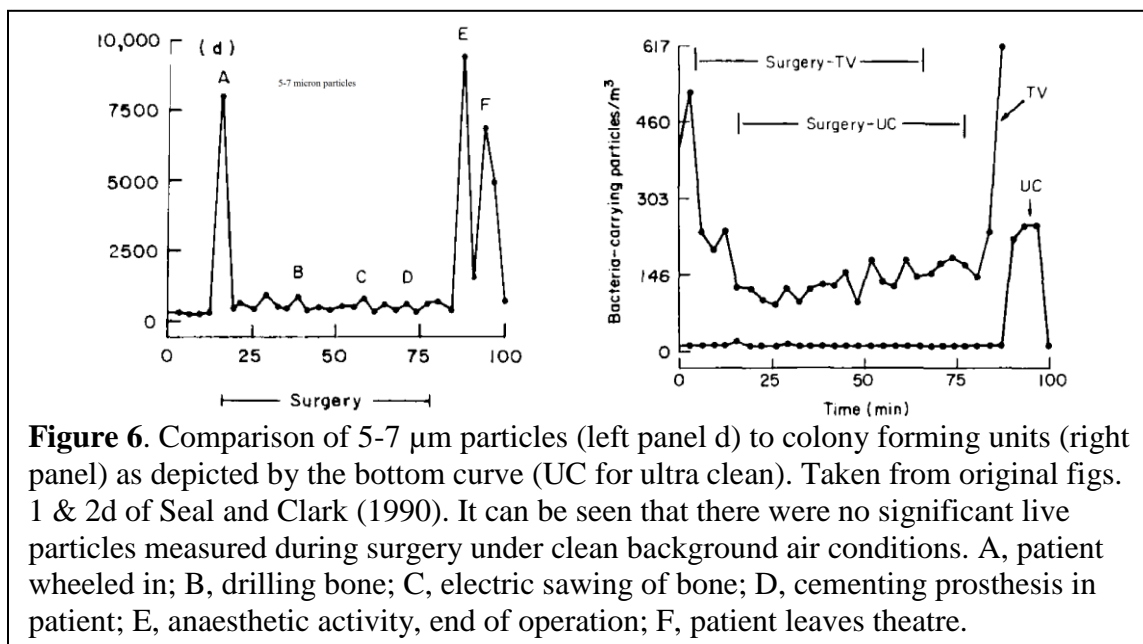


Figure 5. CFU count as a function of 10 μm particulate count. The solid line shows the model-predicted CFU/ m^3 using 10 μm particles/ m^3 . The dashed lines show the 95% prediction interval. From Stocks et al. (2010). Compare this to figure 7.

operating rooms. Live particle counts were measured on impaction agar plates. They concluded that microbiological and particle counting data did not correlate (Spearman correlation coefficient = 0.06, $P = 0.6$). Thus it was suggested there was no reason to replace microbiological sampling with particle counting for routine evaluation of microbiological contamination in conventionally ventilated operating theatres. It is important to note that the appropriate statistical test was used to determine correlation of a phenomenon determined via two separate techniques. Their results confirmed observations made previously by Jalovaara and Puranen (1989).

However, the work of Seal and Clark (1990) was in contradiction, thus it is necessary to revisit their data as shown in figure 6. Replicated here is a composite of their

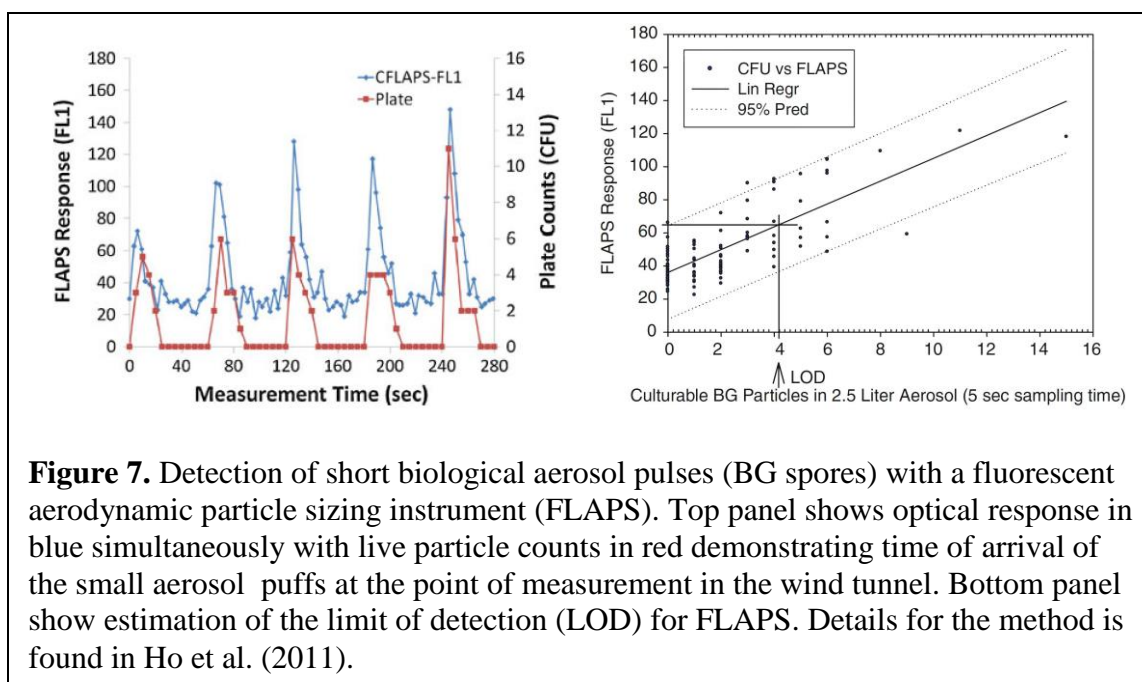


original figures 1 and 2d lined up one on top of the other to show similar time scales with surgical events denoted as A, patient wheeled in; B, drilling bone; C, electric sawing of bone; D, cementing prosthesis in patient; E, anaesthetic activity, end of operation; F, patient leaves theatre. Unsurprisingly the turbulently ventilated (TV) experiment demonstrated high levels of live particle contamination with spikes uncorrelated with particle counts. Under ultra clean air (UC), there was little to no live particles except towards the end of the whole operation. Viewed this way, it is difficult to conclude a correlation between light scattered particles and live agents generated from surgical activities. Speculatively, if the data set from panel 2d (particle count 5-7 μm) were compared to that of UC (lower panel ultra clean live particles), it would appear unlikely to yield a positive significant Spearman correlation test.

In ambient atmosphere there are dissolved organic polymers released by phytoplankton and bacteria that abiologically self-assemble in surface ocean waters into nano-to micro-sized gels containing polysaccharides, proteins, lipids and other components. Aller et al. (2017) using a cascade impactor, collected size-fractionated aerosol particles from ambient air and from freshly generated sea spray in the western North Atlantic Ocean. Via spectrophotometric methods it was possible to quantify the polysaccharide-containing transparent exopolymer (TEP) and protein-containing material in these particles. The results demonstrated that both TEP and protein-containing material in surface ocean waters are aerosolized with sea spray. Their particle size ranged from $<0.18 \mu\text{m}$ to $>5 \mu\text{m}$ in diameter. The larger particles $>5 \mu\text{m}$ may contain microorganisms, other cells and/or fragments. This example illustrates the diversity of aerosol particles found in ambient air.

Ambient air contains a large number of contaminants and non biological or dead particulate material being a major part (Jones 2002). Lessons learned from sampling atmospheric particles (Yee and Ho. 1990) indicated that optical particle counting cannot distinguish live agents from contaminants. Flow cytometry studies provided insights into measuring fluorescent metabolic molecules in single cells as an indication of viable characteristics. This led to development of a UV laser instrument that measured fluorescence of viable biological particles in air (Ho 1996). Later a commercial instrument was made (Hairston et al. 1997) that met military specifications for real time biological threat detection using custom alarming software (Ho 2002, 2014). UV excited fluorescence as a measure of cell viability was later confirmed with growth and viability stain methods (Laflamme et al. 2005).

Anticipating the need for operating room monitoring, Ho et al. (2011) measured biological pulses of *Bacillus subtilis globgii* (BG) spore aerosol introduced into a clean air wind tunnel to simulate spikes of contaminants produced by surgical procedures. Three second aerosol pulses were introduced upwind of the detection port using an airbrush containing a liquid BG suspension. Real time fluorescent signals were monitored via a FLAPS fitted with a virtual impactor to concentrate the dried aerosol stream prior to measurement. At the same time, live particles were impacted on to a slit sampler loaded with a rotating nutrient agar plate. Highlight of the results can be seen in figure 7 where the left panel shows optical response in blue simultaneously with live particle counts in



red demonstrating time of arrival of the small aerosol puffs at the point of measurement in the wind tunnel. The right panel shows estimation of the limit of detection (LOD) for FLAPS, in this illustration, it shows a value of about 4 live particles per 2.5 liter of air. This represents a measuring time of 5 seconds.

Cristina et al. (2012) studied the biological aerosol environment of a HEPA filtered operating room with a two channel ($>0.5 \mu\text{m}$ and $>5 \mu\text{m}$) light scatter particle counter in parallel with live particles impacted on agar growth plates. Measurements were performed when instruments released surgical smoke, for example an ultrasonic scalpel. The results did not reveal any statistically significant correlation between microbial loads and particle counts for either of the particle diameter groups. They concluded that particle measurement could not replace live particle measurement. Viewed in conjunction with the previous papers by the other authors, it becomes clear that particle measurement is subject to nonspecific interference and so not a useful gauge for live contaminant monitoring.

III. Aerobiology Applied to the Bair Hugger System

A. Survival of Microbes within the Bair Hugger

The interior of the Bair Hugger warming unit and hose is not an environment that is favorable for bacteria. Referring to figure 1, it can be deduced that in air, the half life for *S. aureus* is about 5 hr and *Klebsiella pneumoniae* is 1.5 hr. The former is a Gram +ve encapsulated aggregate whereas the latter is a Gram -ve fragile organism similar to *E. coli*. Although *Klebsiella pneumoniae* produces EPS, it does not appear to confer very robust resistance to air exposure. It has been demonstrated that Gram -ve organisms are more susceptible to chemical insults (Majchrzycka et al. 2015). In the air all

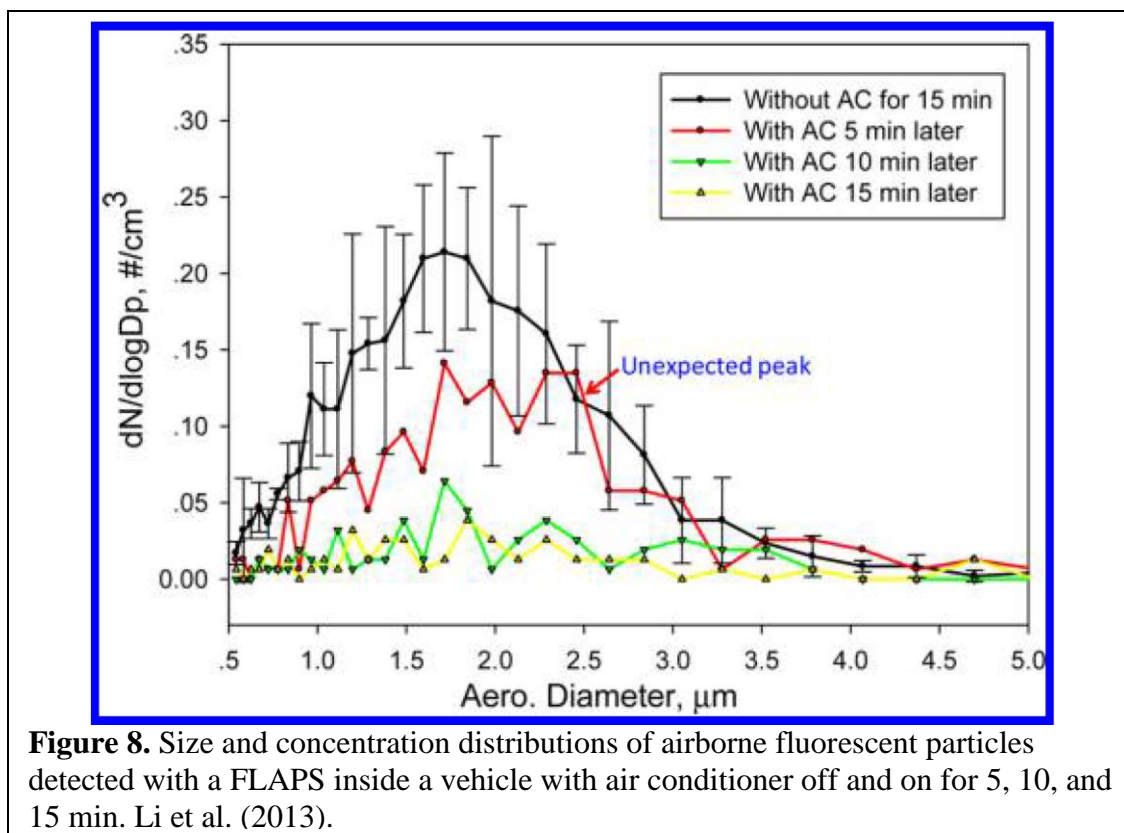
microorganisms show different decay rates dependent on water activity and extracellular matrix (Mugnier and Jung 1985). Their studies showed that at 50% RH at room temperature, a well preserved soil bacterium *Rhizobium japonicum* had a half life of about 6 days. These examples suggest that given the interior environment of the Bair Hugger, most bacterial contaminants will decay rapidly just by being left alone. Moreover, it is unlikely these conditions can promote cell replication.

It has been shown that bacteria adhere to surfaces via EPS, protein based contaminants and electrostatic interaction (Hauslich et al. 2013). Wei et al. (2014) measured strong charge characteristics present in both Gram type bacteria. Metal with rough surfaces and plastics are ideal platforms for adherence. Asbach et al. (2016) summarized particle adherence in plastic tubing, being most significant for polyethylene and polytetrafluoroethylene (Teflon) whereas electrostatic losses in polyvinylchloride (Tygon) were lower. Given that in interior of the machine in question consists of metallic and plastic surfaces, particle loss due to non specific adherence may be expected. Unless subjected to physical disturbance, few if any particles will be emitted during operation.

Temperature affects survival of bacteria in air. For example, in the food industry, 70 degrees C for 2 min has been recommended as sufficient for killing *Staph. aureus* in moist conditions (Kennedy et al. 2005). The Bair Hugger delivers warm air in a dry environment; therefore, the contaminant kill rate may be enhanced above room temperature.

B. Bair Hugger Filtration of Microbes

Before discussing the performance of Bair Hugger filtration, it is instructive to explore how air filtration systems work in other applications. Li et al. (2013) wanted to measure the biological aerosol content in a car passenger compartment equipped with air filters integral with air conditioning. The experiment was carried out in a large city, presumably representing the worst case scenario in terms of particulate contamination. Viable particle estimate was done with a FLAPS located inside the car. Fluorescent particle size distribution spectra were recorded at various times after the air conditioning system was started. As shown in figure 8, it can be seen that after 10 minutes of operation, viable particles dropped to a very low level and after 15 minutes, few particles were present over the size distribution measured. Note the reduction of fluorescent particles in the 1-2.5 μm size range, as this will be discussed further below. Similarly, to test the effectiveness of different car filtration systems, Vonberg et al. (2010) measured number of bacteria and fungal spores by impaction in a high flow air sampler and measured growth on agar plates. They concluded the air conditioning systems in all test cars significantly improve the quality of air inside the cabin. These demonstrations illustrate effective filtering and monitoring of the presence of viable biological contaminants performed under real time situations. Considering the added complexity of the heating and cooling exchangers in car air handling designs that may present more opportunities for microbial lodging, it is remarkable that clean air could be maintained. In the simpler internal design of the Bair Hugger, it is expected that filtration would be appreciably more effective.



Having seen the car biological filter performance characteristics, the question arises, how would this compare with the efficiency of the filtration system in the Bair Hugger? Pui et al. (2008) measured the filtration systems of two car models for nanoparticle filtration efficiency. Private communication with one of the authors (Qi) revealed the car filters may have MERV estimated at 4. From this, it can be extrapolated that the Bair Hugger MERV 14 filters would perform significantly better than the car air handling systems when challenged with live biological aerosols.¹

A MERV 14 filter will effectively remove bacteria and bacterial aggregates (Kowalski 2011). As noted above, fluorescent particles in the 1-2.5 μm size range are representative of naturally occurring biological aerosol, and biological agents travel as aggregates of about 2-6 μm aggregates. According to table 8.2 of Kowalski, a MERV 14 filter will remove *Staph. aureus* with 97% efficiency, and *Enterococcus* with 99% efficiency. Thus, Kowalski's point on page 124 of HEPA filters being “overkill” while quoting Luciano is quite valid. This neutralizes the Reed et al. (2013) suggestion that the Bair Hugger should have inlet filtration upgraded to HEPA quality (99.97% efficient) to prevent microbial ingress.

Bacteria adhesion to surfaces can enhance filtration efficiency of bioaerosols. The same mechanisms that cause bacteria to form biofilms on joint implants can make them

¹ I have reviewed documentation from 3M (EX360-001) confirming that the Bair Hugger filters meet MERV 14 requirements.

more susceptible to filtration. Yuan et al. (2017) discussed the behavior of bacterial adhesion to a variety of surfaces, including filters. The “stickiness” of bacteria enhances the capability of the Bair Hugger MERV 14 to filter them out vs. inanimate particles in the same size ranges.

The study by Bernards et al. (2004) on *Acinetobacter baumannii* particles revealed that microbes transported via large aggregates or dust particles can be trapped in Bair Hugger filters. The paper does not provide evidence from which to conclude that any viable *A. baumannii* organisms passed through the filter and out of the Bair Hugger system. Based on the above discussion, I would expect all such organisms to be effectively filtered by the Bair Hugger’s MERV 14 filter.

C. Research on Bair Hugger and Microbes

In 2007 and 2008, Mark Albrecht, an employee of a competitor to the Bair Hugger, conducted experiments in 4 Minnesota hospitals to detect the presence of microbes in Bair Hugger devices (Albrecht Exhibits 1, 2, and 3, Augustine Exhibit 8). Albrecht took swab samples from the inside of Bair Hugger hoses and cultured some CFUs. This finding is not surprising, because the Bair Hugger is not treated as a sterile device. Albrecht also used an optical particle counter to count particles emitted from the end of the Bair Hugger hose, and found an increase relative to particle counts in OR ambient air. But an optical particle counter cannot distinguish between viable bacteria and inanimate particles of the same size. Finally, Albrecht used an impaction sampler and a filter fitting to try to detect viable bacteria in the airstreams of the Bair Huggers sampled. Albrecht found essentially no CFUs in the Bair Hugger emissions. This research was never published or mentioned in any of Albrecht’s later publications implying that the Bair Hugger is a source of pathogenic microbes.

Albrecht’s negative findings in 2007-08 were consistent with literature that preceded his experiments. Zink and Iaizzo (1993) measured airborne bacteria using agar plate method and concluded that convective warming therapy did not increase the risk for airborne bacterial wound contamination in the operating room. Similarly Dirkes and Minton (1994) summarized that their study showed that convective warming with a filter rated up to 5 microns in size was effective in preventing the transmission of bacteria through the system.

Huang et al. (2003) sampled biological aerosol with a Biotest RCS centrifugal air sampler and Biotest Hycon agar strips. Surprising, the results showed a significant decrease in the colony counts at the end of surgery as compared with the beginning. It would appear the Bair Hugger filtration functioned as a room cleaning mechanism rather than as a problem like others might have implied. More importantly, none of the patients developed postoperative wound or prosthetic infections during a 6-month follow-up period.

Finally, Moretti et al. (2009) employed the Active Surface Air System (SAS) sampler which impacted particles on a single agar plate. By inspecting figure 2 in this

paper, it can be seen that the bacterial population decreased during the use of the Bair Hugger, again implying that the device was serving as a filtration system.

Albrecht and colleagues' published papers were based on swabbing and particle counts to suggest the presence of microbes in the Bair Hugger airstream. Based on the data presented in the Albrecht et al. (2009) paper, it was difficult to extract useful information from optical particle counts. In their figure 1, they were attempting to determine filtration efficiency but the methodology described for this work was not consistent with defined standards for achieving this aim. On their second aim, it would appear the authors were not familiar with microbiological concepts as the experimental design had no control. Samples were analyzed by outside contractors. The drawback in such execution is that interpretation of results can be difficult to comprehend and improvement in experimental design can be retarded or handicapped. Thus guessing at what they actually did, cotton swabs were used to obtain bacterial particles attached to the Bair Hugger hose. The data shown in their table 2 represented yes/no approach which suggested the authors were expecting the instrument to have possessed complete sterility or total worst case contamination. This is an unrealistic approach to measure any instrument for contamination. Further, it was unclear if the bacterial material reported in their results represented infectious agents common to most hospitals. A good example of the proper way to measure filtration efficiency for biological agents can be found in the paper by Majchrzycka (2014).

It is interesting that the authors of Albrecht (2009) included this quote in their discussion that described the work of Tumia and Ashcroft (2002): "Research in ultra-clean operating theatres is limited to a single orthopaedic study in which forced air warming resulted in elevated microbial counts over the surgical site. However, the increase in contamination was deemed to be less than that resulting from the movement of personnel, and did not exceed recommended bacterial levels."

Albrecht et al. (2011) measured submicron particles (figure 2 original paper) using two condensation particle counters (models 3772 and 3782 from TSI). It was not explained how measuring submicron sized particle helped this study. Again, microbial samples were done by outside contracting. Not surprising, it was concluded that Pearson correlation coefficients indicated a lack of correlation between generated particles with internal levels of microbial content from the machines tested. Bacteria counts were shown in their figure 5. Discounting the numerous outliers (primarily noise), the significant counts were in to 10 to 20 CFU range. These numbers do not appear concerning.

However, to those not familiar with environmental microbiology, these numbers may elicit mild concerns, especially in most of Albrecht's papers where surface swabs were routinely used to suggest safety issues. Clearly, there is a need for examples of swab samples of bacterial presence yet they do not represent a safety concern. Yu et al. (2016) wished to examine what species of bacteria recolonize the surgical site after total knee surgery. They designed a randomized controlled trial where 16 patients were allowed to shower at 2 days postoperative and of patients who were asked to wait until 2 weeks later

before showering. Culture swabs of skin adjacent to the incision were taken before, just after incision closure, at dressing removal, and at 2 weeks postoperatively. Bacteria types were identified and compared between the two groups. Shown here is the comparison of organisms cultured at 10-14 days later:

Microorganisms	No Shower	Shower
Staphylococcus aureus	0	2
Staphylococcus epidermidis	8	9
Propionibacterium acnes	1	2
Other gram positives	12	11
Fungus (Rhodotorula)	1	0
Gram negatives	3	2

Note it was not unusual to detect potential pathogens on the skin in both groups, especially *S. aureus*. Their results revealed no difference between the groups in rate of colonization or bacterial type and no patients developed infection. To emphasize the relevance of this work to the present inquiry, the presence of potential pathogens via swab samples near the site of surgery is no cause for alarm. As can be seen, the swab method is not a quantitative technique, rather it indicates more or less as a yes/no suggestion.

The authors of Albrecht et al. (2011) did not explain why they elected to measure submicron particles. This group of particles is not associated with those of biological origin; it is not surprising there was lack of correlation with swab data. However, their intention became clear in the Reed et al. (2013) paper. By this approach, they could make the claim that the Bair Hugger has a filter efficiency of 64% at 0.2 μm size as discussed below.

Reed et al. (2013) set out to determine filter efficiency by generating NaCl submicron particles at size range of 0.025-0.50 μm and analyzing particles with 2 condensation nuclei counters. The regular larger particle size range was measure by pulling a downstream vacuum at 1274 liter/min (45 CFM). This is close to the Bair Hugger filter testing flow rate of 48 CFM. The composite result was shown in their figure 1 which gave an overall efficiency of 64-90% with wide error bars. It should be noted that this efficiency value is at variance with those published by the manufacturer (RD-TEST-PW-05-286536). Based on the lower 64% value for 0.2 μm the authors proceeded to conclude that this may lead to particle leakage and to a significant buildup of internal microbial contamination in the Bair Hugger. Of course this prediction is highly unlikely as this narrative has established that bacterial aerosols consist of particles much larger than 0.2 μm . The authors make a surprising statement on page 278 under the discussion section as quoted here, “Most FAW blowers were also found to be internally generating airborne contamination downstream of the intake filter. This contamination was emitted into the operating theatre through the FAW hose-end airflow.” Let us reflect on this for a moment; Reed claims that the blower motor produces endogenous non biological particles which end up outside the machine. If this were true, all previous attempts at equating total optical particle measurements with biological contamination must be false.

At least they finally conceded that there was no correlation between blower-generated particles and internal levels of microbial content expressed as CFU for individual swab locations.

In the end, the authors did make a few concessions. For example, they did not identify the Bair Hugger as presenting a direct risk in the OR since the output air does not reach the surgical site and further crediting the coverlet as conferring protection as a secondary microbial filter (Avidan et al. 1997). Also, they admitted to not tracking hospital infections, nor studying the association between machine contamination generation or emission and hospital infection rates.

It has been mentioned that particle measurement is not a valid indication for the presence of biological agents in air. Legg and Hamer (2013) measured particle concentration over the surgical site using an optical particle counter. They concluded that it did not appear that the forced-air warming device itself induced contaminated air directly into the surgical enclosure. The study did not show that forced-air warming increases the risk of infection.

One more example may put to rest the issue of airborne bacteria presence as a false alarm. To provide a perspective of what bacterial exposure people are exposed to daily, Bonetta et al. (2010) analyzed biological aerosol in an office with an air conditioning system. Airborne bacteria were sampled via an air flow impaction on agar growth plates. Their results show bacterial and fungal concentrations in the offices ranging from 50 to 500 CFU/m³. Note in this case, a quantitative measurement was possible because the instrument had a specific sample flow rate. The bacterial species identified in indoor air were mostly *Micrococcus* and *Staphylococcus* genera attributed to human contribution. Other species encountered in indoor air were opportunistic pathogens (*S. haemolyticus*, *S. epidermidis* and *S. hominis*). It was concluded that the heterogeneity of the bacterial species and the low counts reported pose no risk for occupant health.

This example highlights the weakness in the Albrecht et al. (2011) paper where their figure 5 showed non quantities measurements. It would have been more meaningful if they blew air through the hose and measured the aerosolized material. Because they did not measure the flow characteristics of live biological particles coming out of the hose but instead just did surface swabbing, interpretation of the data was difficult at best.

As further evidence that the presence of microbes within the Bair Hugger is not a concern, a recent study by Richard et al. (2017) used ATP bioluminescence swabs to take samples of inside the hose of a Bair Hugger, along with other areas in the operating room that were on a spectrum of dirty to sterile after cleaning. The swabs were then analyzed with the use of the handheld luminometer that measured the amount of bioburden for the given area that was swabbed. The amount of ATP, both microbial and nonmicrobial, was quantified and expressed as relative light units (RLUs). The results were compared with thresholds for contamination set by the hospital industry standard of >500 RLU. It was shown that inside of the Bair Hugger hose interior measured 212.5 ± 155.7 RLU, below

the industry threshold. The authors considered the degree of bioburden as relatively small compared with other OR surfaces. This conclusion confirms that the hose interior is not a haven for bacteria.

Finally, Oguz et al. (2017) assessed the number of airborne bacteria by positioning four agar plates in the OR and two nitrocellulose membranes filters directly on the sterile instrument table. There was no difference for bacterial growth on the plates between the forced air and the non-forced air warming treatments. There was no difference for forced air versus resistive warming for bacterial count on either plate. In a follow-up of all patients until hospital discharge, no infections were reported. This quote from the authors sums up all the concerns regarding the safety of the Bair Hugger: “In our study it was not possible to detect any higher bacterial counts on any plate in the forced air warming group versus the resistive warming group. The study may obviously not be generalized for an overall safety statement on forced air warming, and is primarily applicable in the particular surgical setup. However with class action lawsuits ‘judging’ the scientific question of forced air safety with unsuitable, i.e. legal, means subsequent studies are all the more warranted. Only a large, randomized, controlled trial of forced air warming versus nonforced air warming will help to decide, if patient outcome is influenced by the use of forced-air devices. Until this study has been performed, the hypothesized risks of forced air warming remain unclear. With a multitude of factors influencing a patient's risk for perioperative infection, only this kind of study will be able to answer the question, if forced air warming is a major influence on surgical wound contamination, whose voice can be reliably detected in the large choir of all the other factors, such as transmission via the anesthesiologist's or surgeons hand, skin preparation, sterile surgical technique, duration of surgery, surgical skill, patient-related risk factors such as obesity, diabetes mellitus or pre-existing colonization and inadequate antibiotic treatment among many others.”

IV. Observations on Plaintiffs’ Experts’ Reports

A. Michael Buck

In the Buck report on page 5 the bacteria size range was depicted as 0.3-1.2 μm . This explains his choice in using a Fluke model 983 optical particle counter, but as discussed above, 0.3-1.2 μm is not the relevant size range for detecting airborne viable bacteria.

On page 8 Buck expresses his intention to measure filter efficiency but in his data analysis the necessary calculations were not performed to express the results in a percentage as is conventionally done. All the plots showed raw particle concentration versus a time scale so it becomes difficult to interpret. The particle size distribution was not sorted properly but by inspection of the raw plots, there appeared to be few particles $>1.0 \mu\text{m}$. No attempts were made to measure bacterial aerosol from the Bair Hugger. Buck’s experiment was not designed to detect bacterial aerosol and his results cannot support any inference that the Bair Hugger system emits viable bacteria.

B. Dan Koenigshofer

On page 21-22, Koenigshofer concludes that the Bair Hugger contributes 30,000 cfu/hr by extrapolating cfu measurements from an ASHRAE chart (his figure 7). Based on the filtration discussion above, such an extrapolation is unwarranted. Moreover, no published research has found any increase in bacteria near the surgical site during Bair Hugger use. There is no scientific support for Koenigshofer's extrapolation.

On page 23 of the Koenigshofer report, it was summarized that the Bair Hugger filter performance was inadequate with respect to that of the OR. Koenigshofer fails to appreciate that at MERV 14, this filter is perfectly able to remove any bacterial particle threat as explained in this narrative as well as on page 119 in Kowalski (2011) that describes efficiency of bacterial filtration.

Koenigshofer states in his opinion #4 on page 23 that "[t]he hot air from the Bair Hugger will interfere with the downward flow of clean air from the ceiling diffuser." It is unclear from the statement whether Koenigshofer is referring to laminar or conventional diffusers. However, Gastmeier et al. (2012) cast doubt as to whether laminar flow systems actually reduce infections.

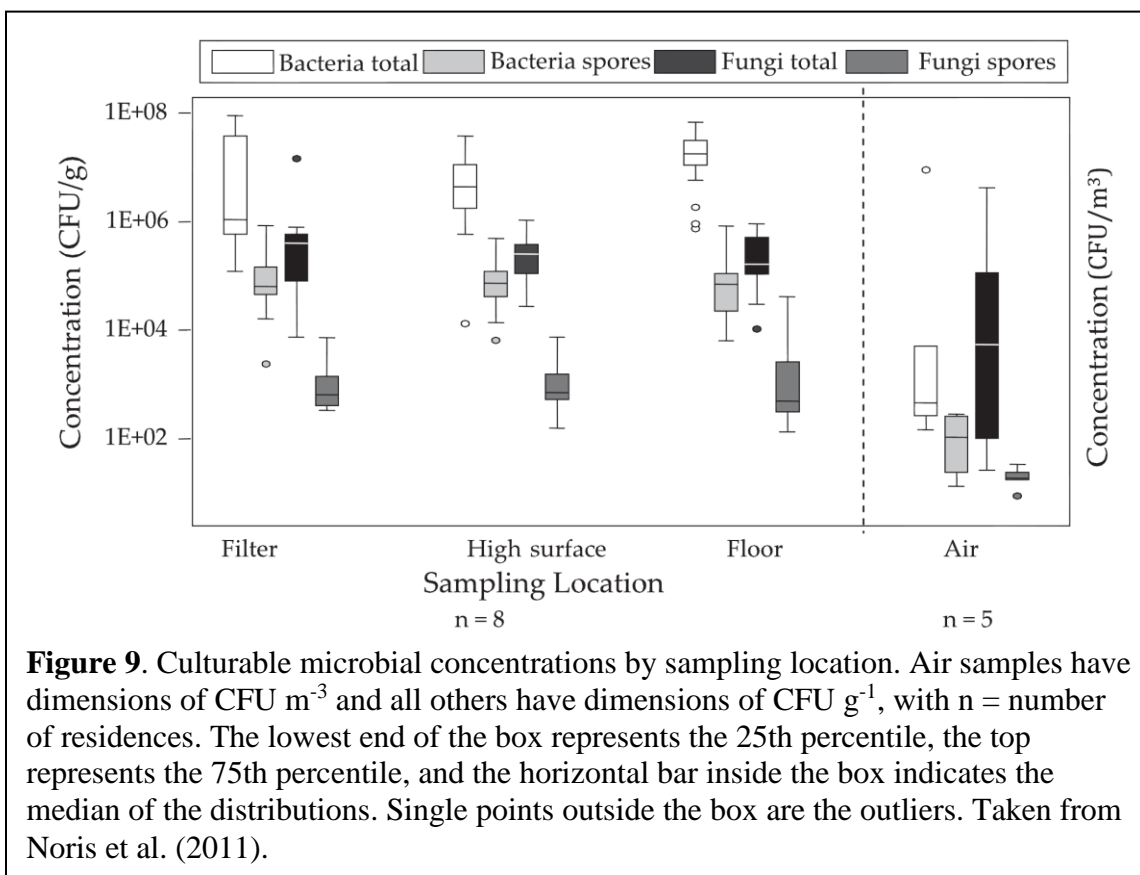
C. Yadin David

In the David report, page 12, it was mentioned that "This dark and warm cavity containing the filter housing and the fan motor can serve as hospitable environment for harboring and incubating colonies of pathogenic bacteria and introducing that bacteria into the flow of air delivered to the patient's blanket." This is a misconception for the interior of the machine in that there is no liquid or moisture content to maintain a growth environment required of microbial populations. Indeed, due to operational air flow characteristics, the drying effect will certainly cause the RH to be low. See discussion on the effect of RH on *Listeria* survival (Zoz et al. 2016). Thus it is unlikely that the interior of the machine will permit bacteria to proliferate.

Critics of the Bair Hugger would lead one to believe that the Bair Hugger and its use in the OR provides a unique setting in which pathogens can proliferate. But the Bair Bugger system can be compared to that in a typical home which has a furnace integrated with cooling and heating coils. Inserted into heat exchanger is a filter that can be purchased from any hardware store. It is common practice to change this filter once a year but most likely, this is not done. Its operation relies on recirculation of air in a closed loop in summer as well as winter. Humans produce biological particles (Balasubramanian et al. 2012) along with dust particles from a variety of sources and the bulk of these end up loosely trapped by the furnace filter. A significant fraction also ends up on the ducting surfaces of the home HVAC system.

The MERV rating for residential application is 3 with a dust spot efficiency <20%. That should remove particles >10 μm . For better residential units the rating is 7 at 24-30% and particles 3-10 μm are eliminated. Yet there are few if any home owners

rushing to upgrade their system to MERV 14 which is rated for 0.3-1 μm particles. Why is it that infectious agents like *S. aureus* are virtually unknown in the home even though the occupants are known carriers (Hospodsky et al. 2012)? Perhaps the furnace filter has been removing most if not all of the material. Indeed, Noris et al. (2011) reported that this is where all the biological materials are trapped. They tested a variety of filter of different ratings for example seven low-efficiency (MERV <5), six mid-efficiency (MERV 5-8) and three high-efficiency (MERV 9-14) filters. Some of their data can be seen replicated in figure 9. As can be seen, the filter samples contained significant microbial content,



having accumulated from the house air recirculation. Similar contaminations can be from the house surfaces, floors and also particles floating in the air. This is not to say that homes and hospitals have the same air filtration requirements. The point, rather, is that harmful pathogens are ubiquitous (we live with them in our homes), and that modestly efficient furnace filters can effectively control them. By comparison, the MERV 14 filters used in Bair Hugger units are highly effective at trapping biological aerosols.

V. Summary Points

A. Particle counts are not a proxy for microbial bioburden.

Ambient air has abundant population of particles of all sizes as illustrated in table 2 taken from Tittarelli et al. (2008). The authors described the particulate content in a

typical city but the note worthiness is that they converted particle number concentration to mass. Students in aerosol measurements routinely express aerosol data as numbers per unit volume, the raw data provided by all optical counters. However, seasoned journal editors usually remind neophyte authors to express aerosol data in mass units.

Channel	Diameter (μm)	Min (10^{-3} cm^{-3})	Max (10^{-3} cm^{-3})	Mean (10^{-3} cm^{-3})	Mean ($\mu\text{g m}^{-3}$)	% of mass
1	0.3–0.5	13 517	507 529	199 027	11.00	21.24
2	>0.5–0.7	1131	256 899	37 403	6.98	13.47
3	>0.7–1.0	247	73 111	7139	3.79	7.31
4	>1.0–2.5	124	29 743	3054	14.14	27.29
5	>2.5–10	9	2146	206	15.93	30.68
Total PM _{2.5}					35.91	69.32
Total PM ₁₀					51.84	100.00

Table 2. Distribution of particle numbers in the five particle size channels, with relative mass transformations from Tittarelli et al. (2008)

Inspecting table 2 will show that the 2.5–10 μm size group has a mean number concentration of 206/cc whereas the mass fraction is about 31% of all particles, much higher than that the other groups. This is due to volume as function to the cube of particle radius. It means a large particle contains much more material than small ones and in biological aerosol it means higher probability of it containing live bacteria within. In addition to this, the size groups represented by 0.3–1 μm are mostly anthropomorphic material serving as noise if one were wishing to measure viable biological content. For this reason, optical particle counting instruments will always yield artifacts in biological aerosol estimation and fluorescence measuring instruments have replaced them (Gosselin et al. 2016).

B. Bacteria do not survive for long periods in air.

People who are susceptible to respiratory infections often move to drier climates for relief. Anecdotal evidence can be a powerful driver as instinctively, low relative air humidity conditions are favored over high RH. Previous studies suggested that fluctuations of RH are known to have lethal effects on cells (Dunklin and Puck 1948). That inspired Zoz et al. (2016) to characterize the impact of RH variations on the survival of *Listeria monocytogenes*, a bacterium persistent on food processing plant surfaces. It was found that controlled ambient RH fluctuations killed the test organism thus could offer new possibilities to control foodborne pathogens in food processing environments and improve food safety. It should be emphasized that this is just one example of how poor bacteria survival in air can be demonstrated.

C. Bacteria travel in air in clumps or on particles much larger than individual microbes.

In this report, the clumping of bacteria in aggregates has been introduced to illustrate how biological aerosol may interact with filtration media. Here, a graphic example may reinforce that message in a striking way. Wainwright et al. (2009) measured cystic fibrosis human cough droplets in a chamber housing samplers that impacted particles on agar growth plates. It was shown that approximately 70% of viable aerosols collected during voluntary coughing were of particles 3.3 μm in aerodynamic

diameter. *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* were cultivated from respiratory particles in this size range. They also used a centrifugal air sampler to study room air and found positive material in cough aerosols. In the current scenario, it is anticipated that any hospital generated aerosol will have similar particle characteristics when considering filtration performance.

D. MERV 14 filtration is effective at filtering bacterial pathogens.

Which brings us to the performance of MERV 14 with respect to aerosol particles that are mostly encountered in hospital settings. Standard charts list this specification: removal of all bacterial particles size within 0.3-1.0 μm . Recall that in a previous example where car filters of MERV 4 could remove submicron particles, it can confidently be predicted that the Bair Hugger filter system can remove biological aerosol from the operating room.

E. Published research shows that airborne bacterial levels do not increase during Bair Hugger operation.

In addition to the negative findings discussed above, it is educational to summarize the Sikka and Prielipp (2014) paper that reviewed the various reports concerning the Bair Hugger. The reports that are most suggestive of a potential contamination issue are listed below in abbreviated format, listing first the lead author and year followed by what they did then the comments by Sikka and Prielipp.

Albrecht 2009. Particle counts; Testing was done without the blanket, which is required for proper airflow. Did not demonstrate that detected particles were bacteria. Author conflict of interest.

Albrecht 2011. Intake filter retention efficiency/performance, airborne particles, FAW colonization; Testing was done without the blanket, which is required for proper airflow. No demonstration of proper maintenance of filters and FAW units. Author conflict of interest

Belani 2013. Bubble count over the simulated surgical site; did not control for room setup. Author conflict of interest

McGovern 2011. Coauthor was employee of conductive warming company. Did not account for age or medical comorbidities. Assumed causation. Did not account for other infection control measures implemented during study period

Legg 2012. Particle counts and temp. over surgical site; did not simulate OR traffic and personnel

Dasari 2012. Temp. at simulated surgical site; Assumed higher temp. at surgical site increases risk of infection. Did not simulate normal OR traffic. Author conflict of interest

Legg 2013. Airflow visualization, drape temp. and particle entrainment; No direct relationship shown between laminar airflow being affected and increased bacteria over surgical site

Reed 2013. Intake filter efficiency/ performance and air path microbial colonization; relied on particle counts rather than sampling of microorganisms from hose-end airflow. High percentage of control swab contamination (50%). Testing was done without blanket

Baker 2002. Cultures of FAW hose and filter; only a single device was tested

Bernards 2004. Cultures of FAW hose and filter; only a single device was tested. The study was a part of an investigation into an *Acinetobacter* outbreak in the ICU

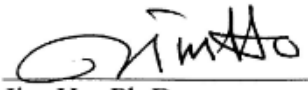
The educational aspect from viewing these papers was that there were so many poorly done measurements. In my opinion these papers were of uniformly low quality. The incidences of interest conflict were also alarming.

It can be assumed that in the OR, the nurse is responsible for implementing the Bair Hugger. Yet the literature on its use is written mostly by personnel who appear to have little to no real day to day contact with the device. So it is refreshing to come across the paper by Kellam et al. (2013) who have “Certified Registered Nurse Anesthetists” credentials and no conflict of interest. The following quote sums up well the real on-the-ground scenario: “The potential that forced-air warming systems may increase the risk of surgical site infections (SSIs) by acting as a vector or causing unwanted airflow disturbances is a concern to health care providers. To investigate this potential, we examined the literature to determine whether forced-air warming devices increase the risk of SSIs in patients undergoing general, vascular, or orthopedic surgical procedures. We examined 192 evidence sources, 15 of which met our inclusion criteria. Most sources we found indirectly addressed the issue of forced-air warming and only three studies followed patients who were warmed intraoperatively with forced-air warming devices to determine whether there was an increased incidence of SSIs. All of the sources we examined contained methodological concerns, and the evidence did not conclusively suggest that the use of forced-air warming systems increases the risk of SSIs. Given the efficacy of these devices in preventing inadvertent perioperative hypothermia, practitioners should continue to use and clean forced-air warming systems according to the manufacturer’s instructions until well conducted, large-scale trials can further examine the issue.”

My opinions are stated to a reasonable degree of scientific certainty. I am being compensated for my work in this case at the rate of \$600 per hour for general consultation and \$750 per hour for testimony. In the past four years, I have testified in

one case, TSI Incorporated v. Azbil BioVigilant Inc., Case No. 2:12-cv-00083-DGCI in the U.S. District Court for the District of Arizona. The materials I considered in arriving at my opinions are cited throughout my report and in my list of References. I reserve the right provide additional opinions and analysis in response to new information pertinent to the issues I have addressed in this report.

June 1, 2017


Jim Ho, Ph.D.

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Jim Ho Career Achievements

His early education took place under British colonial influence, was from [Malacca High School](#), Malaysia and [St. Joseph College in Hong Kong](#). He came to Canada to study at McGill University in Montreal where he obtained a BSc and MSc in microbiology. After obtaining his PhD from the University of Kentucky and since joining the Canadian Department of National Defence in the early 80's, Jim's broad mandate was to develop technologies for the Canadian Forces (CF) to detect biological warfare (BW) agents. To this end, his career can best be summarised chronologically as pre-1990 and post-1990 activities, coinciding with the deployment of equipment for real time detecting [biological threats in the first Gulf war](#). During the first period, his work involved understanding biological aerosol characteristics while the latter dealt with developing a robust biological detection system for the Canadian military and other friendly clients. This brief narrative will highlight significant events for both periods with recent updates.

Biological detectors developed by the US in the 70's and 80's were based on wet chemistry to measure a single parametric "biological component" as detection criteria. These machines failed because they false alarmed or just didn't work reliably. From these lessons, Jim formulated a two step approach to biological detection: A. rapid front end based on [optical light scattering technology](#) and B. slower back end system based on generic UV or fluorescent dye based wet chemistry using variations of existing antibody binding principles. The significance of the scheme is that the optical front end can operate continuously without requiring user attention while the wet chemistry back-end only needs to run on trigger, thus conserving expensive reagents and other resources. Just as important, the rapid responding front end will provide timely information to the military commander for when to initiate protective posture, e.g. donning of respirators. As antibody based clinical methods were relatively well accepted at the time, the major knowledge gap was determined to be in optical methods for differentiating biological aerosols from background material.

Two critical discoveries were made during the early studies. Firstly, it was found that artificial biological aerosols generated as significant threats contained particles mostly in the 2-10 μm size range and secondly, by using a commercial light scattering instrument, these could be distinguished from background aerosols that predominated in the 0.5-1 μm region. Later, it was discovered that biological aggregates depolarised light significantly and this characteristic could be used in remote detection in LIDAR systems (related to RADAR but using laser light). In the middle 1980's, he led a team of scientists from Defence Research & Development Canada (DRDC) at Valcartier and DRDC Suffield in a series of [fundamental LIDAR](#) studies on biological aerosols. Knowledge gained from this period significantly influence fluorescence based long range standoff biological detection directions for Canada and her [NATO](#) allies.

When the Gulf war broke, BW detector systems were deployed at CF locations on Qatar and Bahrain developed from Jim's two step approach, using light scattering techniques and custom software for bioaerosol detection. Canada was the first to deploy such technologies during wartime applications. For having been to the war zone to deploy and train the CF users, he and his team were awarded campaign medals by the Canadian and Saudi governments, a historic first for Canadian civilians. Within multinational agreements, these methodologies were freely shared with allies. Indeed, the two step approach has become accepted as doctrinal for most countries with active BW detection programs.

After the gulf war, in the early 1990's, while searching for better ways to characterise biological particles, Jim discovered that a single spore (typical of anthrax threats), excited with 340-360 nm light, could be induced to fluoresce at wavelength regions representative of important biological molecules like NADH and riboflavin. As these substances could be related to respiratory chain reactions, such fluorescence signals were speculated to reveal important [live biological characteristics](#) and thus mere sand particles would not interfere with bioaerosol measurements. Under his leadership, with the help of outside contractors, the team proceeded to build the first fluorescence aerodynamic particle sizer (FLAPS1) for real time detection of biological aerosols. The success of the instrument was demonstrated at a joint field trial in 1995 where many countries gathered at Dugway Proving Ground in Utah to compete at biological detection hosted by the US Joint Program Office. The Canadian team led by Jim scored first in detection performance. A second-generation instrument was built with better physical attributes ([FLAPS2](#)) and with this instrument, Canada again came in first during the 1996 joint field trial (JFT). Success was repeated in 1997 with a new integrated system that had sample collection built-in. During this time, he also directed the implementation of custom alarm algorithm software with help from contract programmers.

In the later 1990's FLAPS2 technology has been used to manufacture a trigger collector system that served as [CB sentry for the Canadian Forces](#). It was deployed in Operation Determination on HMCS Toronto during the Persian Gulf crisis in 1998. In addition, DND has purchased these biodetection systems and are permanently installed in Canadian frigates that patrol hostile regions of the world. The US Army became the biggest customer of the FLAPS instrument. Jim was lead scientist in designing a third generation (FLAPS3) that became commercially competitive, with world wide sales to NATO and other friendly countries. This product, launched in 2004 was the outright winner of the Technology Readiness Evaluation (TRE) field trials held in June 2003 at Dugway, Utah.

In the global market place, detection of militarily important agents has limited sales scope. However, the ecological monitoring community has adopted the FLAPS technology to determine if live biological agents in natural atmospheric environments contribute to climate change. For atmospheric measurements, this instrument is marketed as [UVAPS](#) by [TSI Inc.](#) in Minneapolis. Similarly, this [original patented technology](#) has been repurposed for biological contaminant monitoring in the pharmaceutical industry ([Biotrack model 9510](#)).

Parallel to developing detection instruments, there was a need to develop reference test and evaluation technologies to determine performance characteristics in the laboratory and the field. Over the years, Jim has developed a field biological challenge and [reference sampling system](#) for testing biological detectors. Some of the DRDC technologies were shared with Dugway Proving Ground when the US was setting up their field capabilities. His [aerosol chamber technologies](#) were also given to the US when they needed the same capabilities to test detector systems in the laboratory. In August and September 2000, the US Joint Program Office on Biological Detection selected DRDC as the location for JFT6. DRDC hosted six participating detection systems from the US.

Jim's aerosol sampling expertise was called to service when the Centre for Disease Control (CDC) in Atlanta needed help in measuring the hazards of anthrax aerosol in the Brentwood Postal Station in November 2001. His team and their aerosol measuring equipment were flown in a Gulfstream IV from Alberta to Washington where they worked in collaboration

with CDC scientists. For the first time, [data from this work](#) demonstrated that anthrax particles could be detected in the building housing the mail sorting machines.

Similarly, in April 2003, Jim and his team were invited to measure the presence of SARS virus at the [Sunnybrook Hospital](#) in Toronto. It was shown that viable SARS viral particles could be captured from the recovery room of a patient who had just been transferred out of the intensive care unit. Up until this time, the medical profession had no suspicion that the recovery room contained infectious agents and that SARS particles could be airborne. The significance of his work in providing proof of SARS aerosol presence in a Toronto hospital was editorialized in the J. of Infectious Diseases (Tong, T.R., 2005. "[Airborne severe acute respiratory syndrome coronavirus and its implications](#)." J Infect Dis 191:1401-2).

Again in April 2004, during the infectious poultry outbreak in the Fraser Valley in BC, he and his team were requested by the Canadian Food Inspection Agency (CFIA) to assist in determining if avian flu virus was spread via the aerosol route. The team from Suffield showed that collected air samples contained live viruses in the chicken barn. Several kilometres from the infected barns, viral particles were also detected using a large volume air sampler. The National Microbiology Laboratory in Winnipeg performed PCR analysis of the viral samples. The results unequivocally demonstrated that avian flu virus was disseminated via the aerosol route.

As recognition for his contributions to biological aerosol research, he had been appointed to the Journal of Aerosol Science editorial board of directors, 1994-97. This journal invited him to co-edit a whole issue on biological aerosols that was published in 1994. Recently, he has been invited to present a lecture on biological aerosol measurement at the 5th International Aerosol Conference in Scotland in September 1998. He was awarded [patent rights to the FLAPS technology](#) which are shared with the Canadian Crown. On 5th February 1999, TSI, a Minneapolis instrument company, concluded a licensing agreement to market FLAPS. In 2001, Dr. Ho, as part of a team that developed a biological detector system, received the DRDC Outstanding Achievement Award. This award recognizes outstanding individual or team achievements that significantly contribute to the Agency's vision. The Outstanding Achievement Award is the Agency's most prestigious award and is given to any DRDC employee or group of employees who have demonstrated unusually high degrees of creativity, expertise, innovation, leadership and dedication.

For having participated in sampling live anthrax at the Brentwood Postal Station in Washington, the U.S. Consul General presented to Dr. Ho and the team certificates of appreciation in 2005. Globally, he stands out as the [leading authority in biological aerosol detection](#) with invitations to contribute [review papers](#) and [book chapters](#). In addition, this has resulted in numerous invitations to speak at conferences as well as military and academic institutions. For example, in 2005, he presented tutorials at AAAR meeting in Austin, TX; EAC conference at Ghent, BE; lectures at the College of Medical Technologist meeting at Camrose, Alberta; talks at University of Tampere, Finland and the Korean military in Seoul, Korea. In 2004 he was invited to present at a workshop in Forschungszentrum Karlsruhe GmbH (FZK), Center for Advanced Technological and Environmental Training (FTU) Karlsruhe, Germany. Over the years, in representing CA at international commitments, Dr Ho has been providing his services to the CBD MOU Test and Evaluation Working Group (TEWG), NATO group on standoff detection and TTCP TP 10 on biological detection. In early 2005, the US Government General Accountability Office (GAO) sent Dr Ho to the UK as part of a team to determine if an X-ray technology was effective in detecting anthrax letters. He was instrumental in devising a method to statistically measure the instrument's performance using simulants.

In recognition for his body of work, Laval University awarded him an adjunct professorship in 2006-present. Also in 2006, the University of Minnesota appointed him to present biological aerosol lectures in their annual [aerosol short course](#). On March 22, 2007, he was invited to present a lecture on the units of measure for biological aerosols to the US National Academies, Keck Center, 500 5th Street, NW, Washington, DC. His recommendation that the unit of measure, agent containing particles per liter of air (ACPLA) be qualified by incorporation of aerosol size data was accepted in a [2008 publication](#). In 2007, he was invited to present the L M Fingerson /TSI Inc. Distinguished Lecture Series hosted by the University of Minnesota.

Since retiring from Suffield in 2012, he has been active as part of a legal team in a lawsuit. A Phoenix Arizona company has attempted to reproduce a copy of the FLAPS instrument in violation of several patents. In the mean time, he is also [consultant to TSI](#) in development of new instruments for the pharmaceutical industry. He is active in collaborating with colleagues from [Waterloo University](#) and [Laval University](#) in developing methods to detect infectious flu aerosol that affect humans.

Having travelled and worked with colleagues from around the world, he has made friends from most of Europe, Australia, America and Asia. In the end, this connection is the most valuable legacy of his remarkable career.

Education and Employment Background

1968 BSc in Microbiology, McGill University
 1971 MSc in Microbiology, McGill University
 1977 PhD in Microbiology, University of Kentucky
 1977-79 postdoctoral research at the University of Windsor
 1980-81, Instructor in Microbiology, St. Clair College, Ontario
 1982-2012, Defense Scientist, DRDC Suffield, DND, CA
 2012-present, consultant to TSI Inc, MN

A comprehensive list of scientific publications available by request or via Google Scholar “jim ho biological aerosol”.

Jim Ho, Ph.D.

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